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# **Control of Foodborne Pathogens and Wholesomeness of Irradiated Food**

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## ABSTRACT

Food can be treated effectively with ionizing radiation doses of less than 10 kGy to eliminate foodborne pathogens. Since these products are not sterile the process can be described as a pasteurization treatment. But unlike thermal pasteurization, there is very little increase in the temperature of the product and the process is sometimes called "Cold Pasteurization." Vegetative bacterial foodborne pathogens, such as *Campylobacter jejuni*, *E. coli* O157:H7, *Listeria monocytogenes*, *Salmonella* spp, and *Staphylococcus aureus*; protozoans, such as *Cyclospora cayetanensis* and *Toxoplasma gondii*; and nematodes, such as *Trichinella spiralis* can be killed by irradiation pasteurization. Irradiated foods have been demonstrated to be wholesome and nutritious.

## INTRODUCTION

Radiation pasteurization when used with proper food processing, storage, preparation, and serving techniques greatly decreases the probability that foodborne pathogens associated with meat, poultry, and other foods will reach the consumer. Stated another way, radiation pasteurization will save lives.

The U.S. Food and Drug Administration has approved the use of accelerated electrons with energies of up to 10 MeV, gamma rays from the isotopes  $^{60}\text{Co}$  and  $^{137}\text{Cs}$ , and X-rays (Bremsstrahlung) with energies up to 5 MeV for the treatment of foods (21 CFR Part 179.26) (<http://www.access.gpo.gov/narn/cfr/cfr-table-search.html>).

Even though the photons and accelerated electrons are highly energetic, the total amount of energy transferred to the foods during radiation pasteurization is small. The international term used to describe the radiation dose absorbed is the Gray (Gy), which is equal to 100 rads or 10,000 ergs per gram. Ten kGy (10,000 Gy) is equal to 1 megarad (Mrad). In general, doses below 10 kGy are pasteurization doses and doses above 10 kGy are sterilization doses. A kilogray (kGy) is equal to 1,000 Gy and would increase the temperature of water  $0.24^{\circ}\text{C}$  or  $0.43^{\circ}\text{F}$ . Since typical radiation pasteurization doses are in the order of 4 kGy, the temperature increase of a typical meat would be only  $0.96^{\circ}\text{C}$  or  $1.72^{\circ}\text{F}$ . It is for this reason that the process is some times called "Cold Pasteurization." Radiation pasteurization may be compared with the energy input for thermal pasteurization of milk, which requires that the temperature of the milk be raised to  $62.8^{\circ}\text{C}$  ( $145^{\circ}\text{F}$ ) and held there for 30 min.

When either a photon or an electron is absorbed within a product, ionization occurs. There are no fundamental differences in the process of ionization resulting from electron,  $\gamma$ -ray, and X-ray irradiation. When a photon is absorbed, an electron is generated. The action can be described as direct when the immediate result of the ionization is the inactivation of microbial replication or an immediate effect on a critical molecule. The reaction is indirect when inactivation results from interaction with free radicals generated by ionization of water or cellular components. Since approximately 70% of bacteria and most foods is water, at temperatures above the freezing point membrane damage and disruption of critical biochemical reactions will account for approximately 70% of the inactivations.

We tend to think that the irradiation of foods to control foodborne pathogens is a relatively recent technique, but, in fact, the German scientist Minck (1896) hypothesized that the newly discovered Röntgen ray might have potential for therapy of bacterial disease. Green (1904) discovered that radium emanations could inactivate several bacterial pathogens, including *Staphylococcus aureus*, *Streptococcus pyogenes*, *Salmonella typhi*, *Mycobacterium tuberculosis*, *Vibrio cholerae*, and *Bacillus anthracis*. Gillet (1918) was issued a U.S. Patent for a device using 16 Röntgen tubes for the X-ray treatment of organic materials, including pork to inactivate trichina. Research on the use of ionizing radiation accelerated following World War II, and some of the current approvals for the irradiation of foods in the United States date to 1963 (Table 1). The most recent is the approval of the irradiation of red meats (domesticated mammalian species, including porcine, ovine, equine, and bovine) to a maximum dose of 4.5 kGy

when non-frozen and 7.0 kGy when frozen. The importance of the differentiation between the frozen and the nonfrozen states will become apparent from the data that will be presented below.

## **RADIATION SENSITIVITY**

The sensitivity of microorganisms to ionizing radiation is affected by a number of factors, including the size of the organism or particle, the complexity of the metabolic and replication mechanisms, and the ability of the organism to repair damage to DNA. In general, the relative sensitivity of microorganisms to ionizing radiation is protozoans > vegetative bacterial cells > spores > virus > prion. The smaller the organism the lower the probability of a direct hit by the radiation. The phase of growth of bacterial cells usually affects their sensitivity to ionizing radiation. Log-phase cells are much more sensitive to radiation than are stationary-phase cells, which in turn are more sensitive than spores (Figure 1). The reason for the great sensitivity of log-phase cells to ionizing radiation is the maximization of both the amount and rate of replication of DNA. The spores are much more compact and dryer than vegetative cells and much more resistant to both ionizing radiation and heat. The past history of an organism may alter its resistance to both heat and radiation, possibly by the activation of better or more efficient repair mechanisms. An example is the adaptation of *E. coli* O157:H7 to acid in apple juice (Buchanan et al., 1998). Bacterial cells will tend to be more sensitive to ionizing

radiation in an aqueous suspension than on meat because many of the components of the meat will compete for the free radicals generated when ionization occurs.

### **INFLUENCE OF TEMPERATURE, pH, AND FOOD ADDITIVES**

Irradiation temperature affects not only the rate of inactivation of the microorganism, but also the reactions that may alter the sensorial characteristics of a food. In part, this can be explained on the basis of the expected decrease in chemical reaction rate as temperatures decrease. However, the most reactive product of the radiolysis of water, the hydroxyl radical, gradually loses its mobility as temperature decreases, until it becomes almost completely immobilized at about  $-60^{\circ}\text{C}$ . At that point the temperature response curve is flat and direct-hit radiation inactivation kinetics of microorganisms predominates (Figure 2). The amount of water surrounding an organism influences the rate of inactivation because of its ionization and the secondary reactions associated with those radicals. Food additives, especially antioxidants, may compete with the microorganism for the free radicals. Several other factors, such as the pH of the product and the atmosphere during and after processing, may alter microbial sensitivity to ionizing radiation. These same factors plus possible interactions with the packaging materials may alter the effects of radiation on the food itself.

## POULTRY, RED MEAT, AND SPICES

Poultry pasteurization is limited to a minimum dose of 1.5 and a maximum dose of 3.0 kGy (Table 1). The precooked, shelf-stable steak used by U. S. astronauts is sterilized with a minimum absorbed dose of 45 kGy *in vacuo* at -30°C. This dose (45 kGy) is based on a determination of the dose required to eliminate  $10^{12}$  spores of *Clostridium botulinum* from meat (Table 2). This process is equivalent to thermally processing canned foods. The only existing approved radiation sterilization dose for domestic products is for spices, with a maximum dose of 30 kGy (Table 1). A considerable amount of spice is sterilized by treatment with either ethylene oxide or ionizing radiation. Most independent radiation processing companies are processing spices and one, SteriGenics is processing large amounts per year in their Tustin, California, plant. Spices are sterilized before use by large food processors because they are typically highly contaminated, and the processors wish to avoid inoculating their products with spoilage and potentially pathogenic organisms. The amount of spice sterilized by ionizing irradiation appears to be increasing. Unfortunately, consumers are not able to directly purchase sterilized spices for use in the home.

## FRUITS AND VEGETABLES

Fruits and vegetables may be treated with ionizing radiation doses up to a maximum of 1.0 kGy for sprout inhibition, disinfestation, and to increase shelf-life either by altering

maturation or by inactivating spoilage organisms. We are learning that products such as raspberries, lettuce or sprouts may be contaminated with pathogenic organisms. Work is currently underway to evaluate the potential of ionizing irradiation treatments to inactivate the protozoan *Cyclospora cayetanensis* associated with raspberries, and the bacteria *Salmonella* and *E. coli* O157:H7 associated with sprouts. The current regulations do not permit the use of ionizing radiation to kill bacterial or protozoal pathogens on fruits and vegetables, nor do they allow the use of a radiation dose that would be adequate to eliminate bacterial pathogens.

## FOODBORNE PATHOGENS

Many of the recent outbreaks of foodborne disease have been associated with the ingestion of poultry or meat products most commonly contaminated with *Campylobacter jejuni*, *E. coli* O157:H7, *Listeria monocytogenes*, *Salmonella* spp, or *Staphylococcus aureus*. The radiation doses required to kill 90% (D-value) of these organisms have been determined on numerous products and are less than 1.0 kGy at an irradiation temperature of 5°C (Table 2). This means that a dose of 1.5 kGy would kill 99.999% (5 logs or 100,000 cells) of *E. coli* O157:H7, which has a D-value of 0.30 kGy, on meat. A dose of 3.5 kGy would be required to kill the same number of salmonella on meat. The D-values for the vegetative cells of genera *Bacillus* and *Clostridium* are similar to those of *Salmonella* or *Listeria*, but the spores are much more resistant. In general, pasteurization doses as included in the current regulations will not effectively control *Bacillus cereus*,

*Clostridium perfringens*, and *C. botulinum* (Table 2). However, several studies have demonstrated that irradiated spores are more sensitive to heat, and similar synergistic results have been obtained with vegetative bacteria such as *Salmonella* (Thayer et al., 1991). The radiation doses required to inactivate the spoilage organism *Shewanella putrefaciens* and the pathogenic protozoan *Toxoplasma gondii* are very low (Table 2). Dubey et al., (1998) using *Toxoplasma gondii* as a model, estimated that *Cyclospora cayatanensis* would be killed on contaminated fruit irradiated to a minimum dose of 0.5 kGy. Since there is no animal model for *Cyclospora*-associated gastroenteritis, and *T. gondii* is believed to be the most resistant of the coccidians, it should serve as an adequate test for the inactivation of *Cyclospora*.

## SAFETY OF IRRADIATED FOODS

The safety of irradiated foods has been demonstrated by microbiological, chemical, nutritional, genetic, and multigeneration toxicological studies of irradiated foods (Thayer, 1994; Diehl, 1990).

Some authors have expressed concern that irradiation of foods may lead to the production of pathogenic microorganisms with enhanced toxicity and resistance to ionizing radiation. First, we need to remember that food is irradiated with doses that are selected to completely eliminate pathogens so that the probability of finding any survivors is extremely remote. Second, the generation of viable mutants capable of competing with normal flora is even more remote. Third, the irradiated foods are not recycled and in most

cases will be subjected to additional processing steps such as cooking before they are ingested. Fourth, the potential for the generation of both bacterial and viral mutants has been studied and found to be remote (CAST, 1986; Cliver, 1969; Maxcy, 1983; Mossel, 1977).

Pathogenic bacteria do not multiply on irradiated foods at rates that are substantially greater than those on non-irradiated foods. Radiation pasteurized foods are not sterile, and significant populations of spoilage organisms survive. The probability of growth and toxin production by *C. botulinum* on irradiated (0.3 kGy) chicken skin is not increased (Dezfulian and Bartlett, 1987; Firstenberg-Eden et al., 1982). It's improbable that salmonella will multiply at a greater rate on irradiated than on non-irradiated chicken (Szcawinska et al., 1991).

The initiation of a large-scale (230,000 broilers, 135,405 kg) study of the wholesomeness of radiation-sterilized, shelf-stable chicken by the U.S. Army in 1976 provided a unique opportunity to compare the effects of freezing, thermal processing (115.6°C), electron irradiation (46 to 68 kGy), and gamma irradiation (46 to 68 kGy) on nutritional quality. The radiation doses are ten times those allowed under current regulations. All of the meat was enzyme inactivated by being heated to an internal temperature of 73-80°C. Protein efficiency ratio values of the meats were not affected adversely. Percentages of amino acids, individual fatty acids, free fatty acid, crude fat, peroxide, riboflavin, pyridoxine, niacin, pantothenic acid, biotin, folic acid, choline, vitamin A, vitamin D, vitamin K, and vitamin B<sub>12</sub> in the thermally-, electron- and gamma-processed meats were not different from those in the frozen control (Thayer, 1990). The

amounts of thiamin in the thermally-, and gamma-processed meats were significantly lower (32%) ( $p < 0.01$ ) than in the frozen control and electron sterilized products. Fox et al., (1989) found that there was a loss of 40% of thiamin from chicken breast meat irradiated to 6.6 kGy at 0°C and then cooked. The conclusion is that there will not be important nutritional changes in irradiated meats.

Nutritional, genetic, teratogenic (causing abnormal development), and multigeneration toxicological studies demonstrated that ingestion of radiation-sterilized chicken is completely safe (Thayer et al., 1987). The Salmonella-microsomal mutagenicity test (a test that is used to determine if mutagenic materials are present) and the sex-linked recessive lethal test in *Drosophila melanogaster* were negative. Teratogenic studies were performed with mice, hamsters, rats, and rabbits fed test meat at 35 or 70% of the total diet on a dry weight basis during the period of maximum organogenesis for the animal. Positive controls produced evidence of teratogenic effects (resorbed embryos and congenital malformations). No test diet induced teratogenic effects. During a chronic toxicity, oncogenicity, and multigeneration study with CD-1 mice, the animals were fed the test diets at 35% of the total diet. During production of two litters from each of the F<sub>1</sub>, F<sub>2</sub>, and F<sub>3</sub> generations there was decreased fertility in mice ingesting the thermally processed diet. There were no significant differences in the frequency of stillbirths, numbers of viable offspring, and survival to weaning in the F<sub>1</sub> through F<sub>3</sub> generations between animals fed irradiated meats and the frozen control. When beagle dogs were fed the test diets at 35% of the total diet, all diets supported growth to maturity. Animals on commercial dog food were significantly lower in weight than those on any of the meat diets. Hematological,

clinical, biochemical, and histopathological findings in the  $F_0$  and  $F_1$  generations were unremarkable with respect to treatment effects. The conclusion was that there were no treatment related effects from feeding the beagles radiation sterilized chicken.

## **CONCLUSION**

Radiation pasteurization, when used in conjunction with proper sanitation, processing, packaging, refrigeration, transportation, cooking, and serving techniques greatly decreases the probability that foodborne pathogens associated with meat, poultry, and other foods will reach consumers.

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Table 1. U. S. Approvals for Irradiated Foods\*

Product	Date	Absorbed Dose
Wheat, wheat flour	1963	0.2 - 0.5 kGy
White potatoes	1964	0.05 - 0.15 kGy
Pork for trichina inactivation	1986	0.3 - 1.0 kGy
Fruits and vegetables	1986	Maximum 1.0 kGy
Herbs, spices, dry vegetable seasonings	1986	Maximum 30 kGy
Poultry	1990 FDA	3.0 kGy maximum
	1992 USDA	1.5 kGy minimum
Red meats	1997 FDA	4.5 kGy nonfrozen and
	USDA Pending	7.0 kGy frozen

\*21 CFR Part 179.26; 9 CFR Part 381.149

Table 2. Dose required to make 90% of bacteria or 100% of protozoans non-viable

Pathogen or Spoilage Organism	Dose kGy	Substrate	Reference
<i>Bacillus cereus</i> endospore	2.78 $\pm$ 0.17	Beef	Thayer and Boyd, 1994
<i>Campylobacter jejuni</i>	0.16 to 0.20	Beef	Lambert and Maxcy, 1984
<i>Clostridium botulinum</i> endospore	3.58 @ -30C	Beef	Anellis et al., 1975
<i>Escherichia coli</i> O157:H7	0.30 $\pm$ 0.02	Beef	Thayer et al., 1995
<i>Listeria monocytogenes</i>	0.45 $\pm$ 0.03	Beef	Thayer et al., 1995
<i>Salmonella</i> spp.*	0.70 $\pm$ 0.04	Beef	Thayer et al., 1995
<i>Shewanella putrefaciens</i>	0.17 $\pm$ 0.01	Beef	Thayer and Boyd, 1996
<i>Staphylococcus aureus</i>	0.46 $\pm$ 0.02	Beef	Thayer et al., 1995
<i>Toxoplasma gondii</i>	0.50	Brain	Dubey et al., 1998

\**Salmonella* spp.: *S. dublin*, *S. enteritidis*, *S. newport*, *S. senftenberg*, and *S. typhimurium*

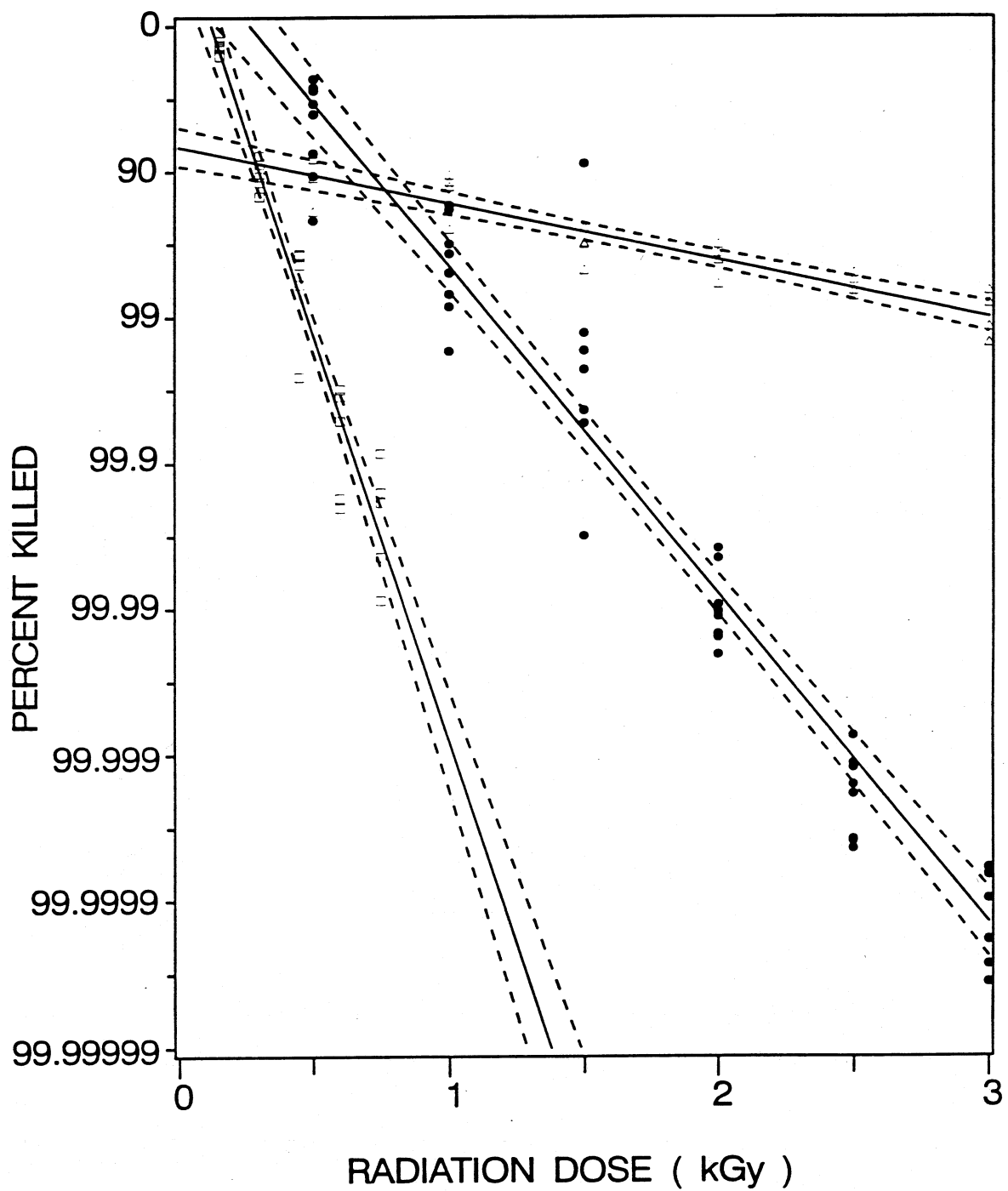


Figure 1. Effect of gamma radiation on log-phase cells, stationary-phase cells and spores of *B. cereus* ATCC 33018 on chicken.

Log-Phase =  $\square$ , Stationary-Phase =  $\bullet$ , Endospore =  $\Delta$ .

Adopted from: Thayer and Boyd. 1994. J. Food Prot. 57:758-764.

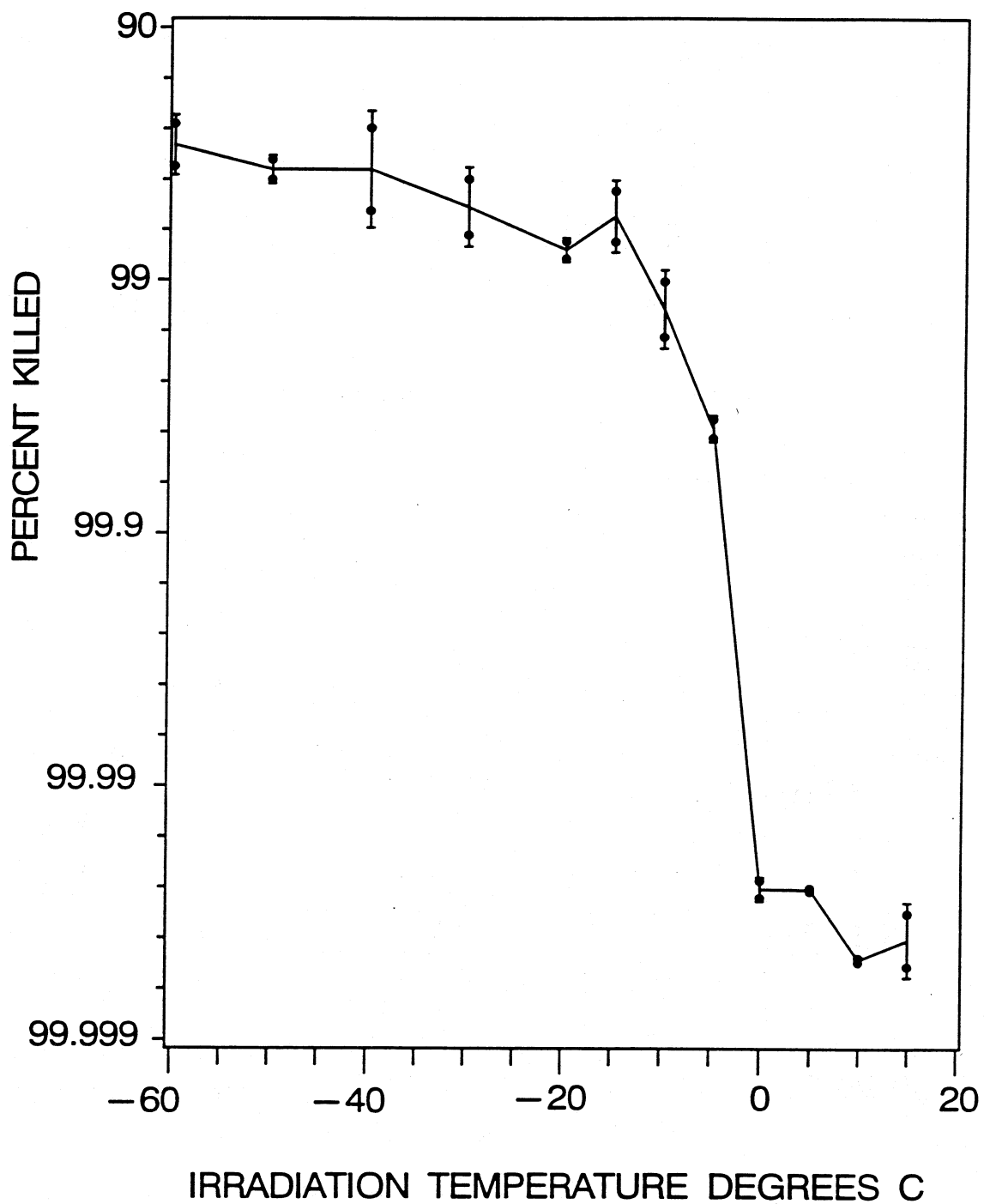


Figure 2. Effect of a radiation dose of 1.5 kGy at various temperatures on *E. coli* O157:H7 on chicken. Adopted from Thayer and Boyd. 1993. Appl. Environ. Microbiol. 59:1030-1034.